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EXPERIMENTAL TERRESTRIAL SOIL-CORE MICROCOSM TEST PROTOCOL

A Method for Measuring the Potential Ecological Effects, Fate, and Transport of Chemicals in Terrestrial Ecosystems

TOXIC SUBSTANCES CONTROL ACT SECTION 4 TEST GUIDELINES

by

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16. ABSTRACT

Effective regulation, enforcement, and litigative action by the United States Environmental Protection Agency's Office of Pesticides and Toxic Substances are predicated upon the evaluation of scientifically valid and environmentally meaningful data on a chemical's potential impact on the environment. Industry must provide EPA with the results of tests designed to forecast potential ecological effects and the probable environmental fate of a candidate chemical substance and/ or mixture (i.e., test substance). To this end, EPA has guidelines to allow effective comparison of results used in the assessment process. For the data to be comparable however, the test must be performed in the same rigorous manner with only slight, yet documented variation allowed. Thus, protocols or standard procedures for performing the tests must be documented for industry to ensure development of comparable data bases. This protocol is one which could be added to a series of tests recently developed as guidelines for Section 4 of the Toxic Substances Control Act. The terrestrial soil-core microcosm is designed to supply site-specific and possibly regional information on the probable chemical fate and ecological effects resulting from release of a chemical substance to a terrestrial ecosystem. The EPA will use the data resulting from this test system to compare the potential hazards of a chemical with others that have been previously evaluated.

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ABSTRACT

Effective regulation, enforcement, and litigative action by the United States Environmental Protection Agency's (EPA's) Office of Pesticides and Toxic Substances are predicated upon the evaluation of scientifically valid and environmentally meaningful data on a chemical's potential impact on the environment. Industry must provide EPA with the results of tests designed to forecast potential ecological effects and the probable environmental fate of a candidate chemical substance and/or mixture (i.e., test substance). To this end, EPA has established a series of standard ecological toxicity and chemical fate test guidelines to allow effective comparison of results used in the assessment process. For the data to be comparable, however, the test must be performed in the same rigorous manner with only slight, yet documented, variation allowed. Thus, "protocols" or standard procedures for performing the tests must be documented for industry to ensure development of comparable data bases.

In order to protect the environment properly and have a realistic appraisal of how a chemical will act in the environment, tests of ecological effects and chemical fate must be performed on complex assemblages of biotic and abiotic components (e.i., microcosms) as well as single species. This protocol is one which could be added to a series of tests recently developed as guidelines for Section 4 of the Toxic Substances Control Act (P.L. 94-469; U.S.C., Section 2601-2629). The terrestrial soil-core microcosm is designed to supply site-specific and possibly regional information on the probable chemical fate and ecological effects resulting from release of a chemical substance to a terrestrial ecosystem. Experience has shown that microcosms are best applied in the assessment process at stages after acquisition of preliminary knowledge about a chemical's properties and biological activity. The EPA will use the data resulting from this test system to compare the potential hazards of a chemical with others that have been previously evaluated.

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TEST PROTOCOL FOR EXPERIMENTAL TERRESTRIAL SOIL-CORE MICROCOSMS

1.0 SCOPE AND PURPOSE

Effective regulation, enforcement, and litigative action by the United States Environmental Protection Agency's (EPA's) Office of Pesticides and Toxic Substances are predicated upon the evaluation of scientifically valid and environmentally meaningful data on a chemical's potential impact on the environment. Industry must provide EPA with the results of tests designed to forecast potential ecological effects and the probable environmental fate of a candidate chemical substance and/or mixture (i.e., test substance). To this end, EPA has established a series of standard ecological toxicity (EPA 1982a) and chemical fate test guidelines (EPA 1982b) to allow effective comparison of results used in the assessment process. For the data to be comparable, however, the tests must be performed in the same rigorous manner with only slight, yet documented, variation allowed. Thus, "protocols" or standard procedures for performing the tests must be documented for industry to ensure development of comparable data bases.

In order to protect the environment properly and have a realistic appraisal of how a chemical will act in the environment, tests of ecological effects and chemical fate must be performed

on complex assemblages of biotic and abiotic components (i.e., microcosms) (Van Voris 1984, 1985; Cairns 1985) as well as single species (Cairns 1981). The protocol presented here is one which could be added to a series of tests recently developed as guidelines (EPA 1982a,b) for Section 4 of the Toxic Substances Control Act (P.L. 94-469; U.S.C., Section 2601-2629). The terrestrial soil-core microcosm is designed to supply site-specific and possibly regional information on the probable chemical fate and ecological effects resulting from release of a chemical substance to a terrestrial ecosystem.

Experience has shown that microcosms are best applied in the assessment process at stages after acquisition of preliminary knowledge about a chemical's properties and biological activity. The EPA will use the data resulting from this test system to compare the potential hazards of a chemical with others that have been previously evaluated.

2.0 DEFINITION OF TEST SYSTEM

2.1 The Terrestrial Microcosm

The terrestrial microcosm or micro-ecosystem is defined here as a physical model of an interacting community of autotrophs, omnivores, herbivores, carnivores and decomposers within an intact soil profile. Specifically, it is an intact soil-core containing the natural assemblages of biota surrounded by the

boundary material. The system includes all equipment, facilities, and instrumentation necessary to maintain, monitor, and control the environment. The forcing functions, e.g., light intensity and duration, water quality and watering regime, temperature, and toxicant dose for the test system, are under the investigator's control. This test system is distinguished from test tube and single species toxicity tests by having a natural assemblage of organisms present, resulting in a higher order of complexity and, thus, the capacity to evaluate chemical effects on component interactions and ecological processes. Like any experimental or standard test system, the microcosm will not duplicate all aspects of the ecosystem under evaluation. Certain features of this test system set limits on the types of questions that can be legitimately addressed. Those limitations are related to scale and sampling, which in turn constrain both (a) the type of ecosystems and species assemblages on which one can hope to gain information, and (b) the longevity of the test system.

2.2 Physical, Chemical and Biological Conditions

The physical, chemical, and biological conditions of the test system are determined by the type of ecosystem from which the test system was extracted and by either the natural vegetation in the ecosystem or the selection of crops for planting. Vegetation and crop selection are constrained and determined by

the size (width and depth) of the soil core extracted.

Boundaries - The boundaries of the test system are determined by the size of the core and the growth space needed for the vegetative component.

Light - Light for the test system can be supplied by artificial lighting in either a growth chamber or greenhouse or can be the natural photoperiod occurring in a greenhouse. If the test is performed in a growth chamber, the daily photoperiod for the microcosm should be at least the average monthly incident radiation (quantity and duration) for the month in which the test is being performed, with a cycle of 12 hours on and 12 hours off.

Water - Water for the test system should either be pre-collected, filtered rainwater from the site being evaluated or should be purified, untreated laboratory water with a known chemical composition.

Soil - The soil for the microcosm should be an intact, undisturbed (non-homogenized) core extracted from a soil type typical of the region or site of interest and be of sufficient depth to allow a full growing season for the natural vegetation or the crops selected, without causing the plants to become significantly rootbound. Disturbances during extraction and preparation should be kept to a minimum.

It should be noted that soil characteristics will play an important role in how the microcosm responds to a test substance further, and, that within-site soil heterogeneity will influence the microcosm response. The approach used in this test system however, is based on comparison of responses among and between treatments rather than on the absolute values measured for an individual microcosm.

Biota - The biota of the microcosm are characterized by the organisms in the soil at the time of extraction and by the natural vegetation or crop species introduced as the autotrophic component. The biota includes all heterotrophic and carnivorous invertebrates in the soil and all soil and plant bacteria, fungi, and viruses.

3.0 CHEMICAL CHARACTERIZATION OF TEST SUBSTANCE AND SOIL

3.1 Information Required on Test Substance

Minimum information required to properly design and conduct an experiment on a test chemical includes the chemical's source, composition, degree of purity, nature and quantity of any impurities present, and certain physicochemical information such as water solubility at 25°C and vapor pressure at 25°C (Laskowski et al. 1982; Swann et al. 1983). Ideally, the structure of the test chemical should also be known, including functional groups, nature and position of substituting groups, and degree of saturation. Octanol-water partition coefficient,

dissociation constant, degree of polarity, and pH shall also be known. Where mixtures are involved or where a significant impurity (>1%) occurs, data must be available on as many components as practicable. However, the octanol-water partition coefficient (P or Kow) stands out as the key value. In combination with other chemical characteristics, log P (Kow) can be used to estimate Henry's Law Constant to predict volatility from water. Soil sorption can be estimated from log P (Kow), the organic matter content, or from those data that suggests a relationship exists. Water solubility can be predicted with some degree of accuracy from log P (Kow) if this value is less than 7.

Several tests may be needed to supply information on environmental mobility and stability. Support information on phytotoxicity, the physicochemical nature of the chemical, its mammalian toxicity or its ecological effects (e.g., species-specific LD_{50}, biodegradability) not only assist in proper design of the microcosm experiment, but also are useful in assessing the fate and effects of the chemical in a terrestrial microcosm. If the chemical is radioactively labeled, the position of the label should be specified.

It is imperative to have an estimate of the test substance's toxicity to mammals as a precaution for worker safety. In addition, hydrolysis or photolysis rate constants should be

known in order to determine necessary handling precautions. When a radiolabeled material is used, normal laboratory techniques for radiation safety provide an ample margin of safety except for chemicals in the "very highly toxic" category (i.e., rat oral LD_{50} <1 mg/kg).

The above information required for a test chemical is considered to be that necessary to handle and apply the chemical safely, as well as provide a perspective from which to interpret the test results. Water solubility, soil sorption and partitioning, and vapor pressure largely will control the physical transport and bioavailability of a test chemical in soil. Water soluble chemicals are likely to move with soil water into the water films surrounding soil particles and root surfaces. Most microbially-mediated biodegradation occurs in the water-containing microsites of soil particles. Plant uptake and bioaccumulation is largely a function of water transfer to roots. In addition, water-soluble chemicals and their transformation products may be leached to ground water. Water solubility of an organic chemical is a function of the dissociation of ionic compounds and the polarity of non-ionic compounds.

3.2 Information Required on Soil

Soil sorption of an organic molecule depends on several properties of the chemical (i.e., molecular size, ionic

speciation, acid-base properties, polarity, and nature of functional groups) and of the soil (e.g., organic matter content, clay content, clay mineralogy and nature, pH, water content, bulk density). Highly sorbed chemicals may displace inorganic nutrient ions from exchange sites in the soil and also may be effectively immobilized, depending on soil pH. Thus, chemicals attracted more strongly to soil surfaces than to water may be very immobile in soil. In some cases, this may render the compound relatively resistant to biodegradation. In other cases, however, immobilization of the compound on soil particles may render it susceptible to extracellular enzymatic degradation.

Compounds with very high vapor pressures (boiling point <80°C or vapor pressure >25 mm Hg) are not suitable for testing in the terrestrial soil core microcosm as currently designed.

4.0 TERRESTRIAL MICROCOSM EXTRACTION AND MAINTENANCE

4.1 Microcosm and Cart Design

4.1.1 The 60-cm-deep by 17-cm-diameter terrestrial microcosm is designed to yield pertinent information about a chemical for either a natural grassland ecosystem (Figure 1) or an agricultural ecosystem planted with a multiple-species crop (Van Voris et al. 1982, 1984, 1985; Tolle et al. 1982; Zwick et al. 1984). The agricultural microcosm is a 17-cm-diameter tube of Driscopipe® containing an intact subsoil core (40 cm) covered by homogenized topsoil (20 cm). Driscopipe® is an ultra-high

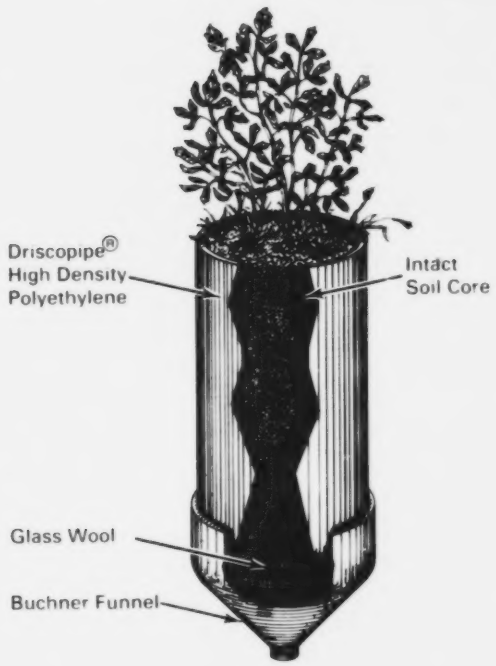

Figure 1. Microcosm Structure and Materials

molecular weight, high-density, non-plasticized polyethylene pipe. It is impermeable to water, lightweight, tough, rigid, and highly resistant to acids, bases, and biological degradation. The tube sits on a Buchner funnel covered by a thin layer of glass wool. The funnel and tube are washed with acid prior to use and are reuseable for future tests.

4.1.2 Six microcosms are typically contained in a moveable cart, which is packed with Styrofoam® beads to maintain a realistic temperature profile (Van Voris et al. 1982, 1984; Tolle et al. 1982) (Figure 2). Cart dimensions are based on the environ-

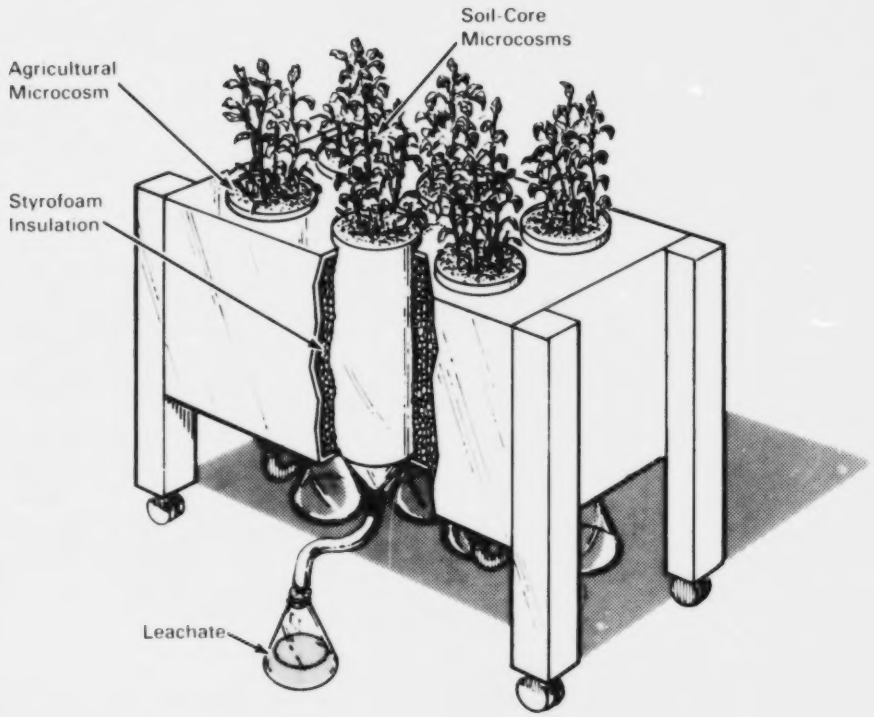

Figure 2. Arrangement of Microcosms in Styrofoam® Filled Cart

mental chamber size or the maneuverability required in the greenhouse. Carts are mounted on wheels so the cart can be rotated in the greenhouse to make exposure to light and temperature more uniform.

4.2 Soil Core Extraction

4.2.1 Soil cores are extracted from either a natural grassland ecosystem or a typical agricultural soil in the region of interest. The intact system is extracted with a specially designed, steel extraction-tube (Van Voris et al. 1982, 1983, 1984; Tolle et al. 1982; Zwick et al. 1984) (Figure 3) and a

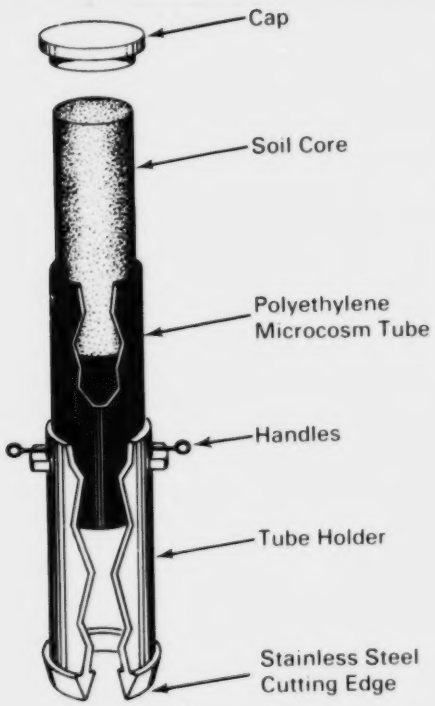

Figure 3. Diagram of Microcosm Extraction Tube

backhoe. The steel extraction-tube encases the polyethylene microcosm to prevent the microcosm tube from warping and/or splitting under pressures created by the backhoe during extraction. For the agricultural microcosm, the plowed topsoil is moved aside and saved. Once the core is cut by the leading edge of the driving tube, it is forced up into the microcosm tube. For the natural grassland ecosystem, the vegetation is clipped before the core is extracted. The soil core microcosm is later removed as a single unit (soil and Driscopipe®) from the extraction tube and taken to the laboratory. For the

agricultural microcosm, the topsoil is backfilled into the upper 20 cm of the microcosm tube. It is recognized that this extraction procedure does disrupt and compress the soil-core to a certain extent; however, the tests are being performed for comparative purposes.

4.2.2 Detailed chemical and physical properties of the soil in the test systems need to be determined using USDA description nomenclature. Information such as pedologic identity, according to the USDA 7th Approximation Soil Classification System, percent organic matter, field capacity, cation exchange capacity, bulk density, macro- and micro-nutrient content, organic matter content, mineralogy, exchange capacity, particle size distribution, hydraulic characteristics, and other important characteristics need to be measured before and after the experiment (Black 1965a,b). The history of the soil, including previous crops grown and pest control and other management practices used, should be documented in order to assist in the interpretation of the results.

4.3 Microcosm

4.3.1 For the natural ecosystem (undisturbed grassland) test system, natural plant cover should be sufficiently diverse to be representative of plant species in the ecosystem of interest. When the agricultural microcosm is used, a mixture of grasses and broad leaves (e.g., legumes) should be included. Two or three species of grasses or legumes that are typically grown

together as an agricultural crop in the region of interest should be chosen. The species chosen must be compatible regarding growth habit and be able to grow to maturity in the small surface area (240.5 cm^2) of the microcosm. In some cases, it may be appropriate to select a grain crop in order to evaluate the uptake of the labeled test substances and their degradation products in grain normally grown for human consumption (Tolle et al. 1983).

4.3.2 The seed application rate should duplicate standard farming practice for the region of interest. Seeds should be planted evenly and covered with an appropriate depth of soil. Similarly, the test substance application form should approximate a reasonable scenario of how the test substance might arrive at the site in question. If the test substance is a solid, one option is to mix it with the topsoil prior to planting, thus mimicking the plowing of an agricultural field prior to sowing seed. Alternatively, it may be dusted on the surface to simulate dry deposition.

4.3.3 Plant tissue analysis should be consistent with those practices that may be region specific. Plants from either the natural grassland ecosystem or the agricultural test unit are harvested at the end of the test period, stored in separate paper bags for each microcosm (Van Voris et al. 1982; Tolle et al. 1982), and air dried, oven-dried, or both soon after harvest to avoid rotting. In the range-finding test, the crop is harvested four

weeks after first exposure to the test substance. In the definitive test, plants may be harvested one or two times during the 12-week growing period and at the end of the test. The definitive test may need to be extended beyond the 12-week test period to accommodate plant species which take longer to reach the desired maturity (e.g., seed production).

4.4 Microcosm Watering and Leachate Collection

4.4.1 Microcosms are watered as dictated by a predetermined water regime based on site history with either Reverse Osmosis (RO) treated water or with rainwater that has been collected, filtered and stored in a cooler at 4°C (Van Voris et al. 1980, 1982, 1983). If comparisons are being made between microcosms and field plots, then parallel watering in both units should be attempted. Care needs to be taken to maintain sufficient water, but over-watering can induce fungal disease and stress. If the test chemical is applied as an aerosol or powder, plants are sprinkled immediately after dosing in order to avoid resuspension of particulates and reduce the potential for cross contamination of dose levels.

4.4.2 Microcosms are leached at least once before and once every two or three weeks after dosing. However, if natural rainfall amounts are higher or lower this should be used to guide selection of a leaching regime. Leachate is collected in acid-washed flasks after excess RO water or rainwater has been added to each microcosm. The 500-ml flask, attached to the Buchner

funnel with Tygon® tubing, is supported by a wooden board fastened under the microcosm cart (see Figure 2). To insure that all test microcosms will leach within a 2-day period, 15% more microcosm soil cores are extracted than are required for the tests. When the microcosms are leached before planting, those which do not leach, leach too quickly, or take longer than two days to produce 100 ml of leachate after the soil has been brought to field capacity are discarded.

4.5 Greenhouse and Growth Chamber Environments

Microcosms in insulated carts or other such devices are kept in a greenhouse or environmental chamber that has temperature and light control. Greenhouse and environmental chamber temperatures are designed to approximate outdoor temperatures that occur during a typical growing season in the region of interest. If the experiment is not conducted in the greenhouse during the normal agricultural growing season, then lights suitable for plant growth on timing devices are used to simulate the photoperiod and intensity typical for the growing season in the area of interest.

5.0 TEST PROCEDURES

5.1 Test Purpose and Assumptions

The purpose of the terrestrial soil-core microcosm test is to determine the fate and ecological effects of a test substance, including transformation products, within a site-specific

natural grassland or agricultural ecosystem. The relationship of fate and effects data from treated versus control microcosms is assumed to be very similar to that from treated versus control field test plots (Van Voris et al. 1982, 1983, 1984). This assumption is supported by the microcosm/field comparisons reported by Van Voris et al. (1982, 1984), Jackson et al. (1979) and Tolle et al. (1982, 1983). The fate and effects from the microcosm test should then be related to either the natural or agricultural ecosystems which have the same combination of soil type, vegetation, crop species, and environmental variables used during the microcosm test.

5.2 Evaluation of Test Substance

5.2.1 Physicochemical information supplied for the test substance (see Section 3.1.1) is used to tailor the general range-finding test procedures to the specific substance. Phytotoxicity and/or bacteriostatic action, if known, should be taken into account in designing the exposure concentrations of the range-finding experiment. Only one concentration above that known to cause at least 50% change in plant growth or 50% change in bacterial growth/respiration needs to be tested. In addition, the lowest treatment level should not be lower than a factor of 10 in soil and 100 in water. These factors are the analytical limits of detectability of the parent compound at the start of the experiment.

5.2.2 The water solubility and soil sorption capacity can be used to determine the appropriate frequency of leachate analyses for the radiolabeled test substance and its transformation products. This same information will also determine the design of the soil sampling procedures for the range-finding test. Chemical structure and any degradation information are used to determine which transformation products to analyze for in the soil, leachate, and plant tissue collected.

5.2.3 As was stated in Section 4.3.2, exposure should approximate a reasonable scenario. The water solubility, dissociation constant(s), and soil pH must be taken into account in determining the formulation of the chemical. The maximum concentration of a chemical in water solution should not exceed half of saturation. However, solubility may be markedly altered by ionization in soil. If the soil pH is such that a more soluble form is likely, the test substance should be adjusted accordingly with either sodium hydroxide or hydrochloric acid prior to introduction into the microcosm. If the pH adjustment to increase solubility is extreme (4<pH>9), chemical and photolytic degradation may be enhanced when preparing the chemical solutions. In all cases, the exposure formulation should be consistent with the hypothetical scenario forming the basis for the test.

5.3 Range-finding Test Experimental Design

The range-finding test should last four weeks from first exposure of the test substance to plant harvest. At the start of the test, the microcosms are dosed (see Section 5.5) with a minimum of five concentrations of the test substance. Three replicate microcosms are used for each of the four or five treatment levels and the controls, resulting in a total of 15 or 18 microcosms. Concentrations typically used for dosing are 0.1, 1.0, 10, 100 ppm, if a hypothetical scenario is not known and, if deemed appropriate, 1000 µg/g of topsoil in the upper 20 cm of the microcosm. The logarithmic scale for dose levels in a range-finding test is suggested by Rand (1980). The bulk density (g/cm^3) of the dry topsoil is used to calculate the exposures. Depending on whether the potential mode of release of the test chemical is likely to be a single accidental spill or a repeated effluent release, either a single, "acute" dose, or a multiple application, "chronic" dose, may be more appropriate. In either case, the total amount of test chemical applied should be equal to five of the recommended test concentrations.

Each microcosm cart, holding one replicate of each of the four or five test concentrations and a control, is moved on a weekly basis in the greenhouse to avoid location induced effects. If no discernible effects are recorded during the range-finding test, no definitive test would be performed.

5.4 Definitive Test Experimental Design

The definitive test lasts for 12 or more weeks from first exposure of the test chemical to final harvest. In setting up the test, either a completely randomized experimental design, randomized block design, Laten-square design or another appropriate experimental design should be considered. If not, the results and analysis may be biased, thus jeopardizing the outcome of the experiment.

At the start of the test, the microcosms are dosed with three concentrations of the test substance. Ten replicate microcosms are used for each of the three treatment levels and controls, for a total of 40 microcosms. The three treatment levels chosen are estimated from the range-finding test data to produce a 20 to 25% change in productivity for each subsequent concentration of the test chemical. In order to reduce analytical costs associated with the fate studies, the ten replicate microcosms in each treatment level are employed as five replicate pairs. Thus, leachate and plant tissue analyses are conducted on the pooled samples from paired microcosms. Productivity data, on the other hand, are analyzed for each individual microcosm. Each cart holds six to eight microcosms (see Figure 2). The microcosms paired for analyses are placed in different carts to insure that all microcosms are housed under the most uniform conditions possible.

Depending on the type of natural vegetation or crop planted, it may be possible to harvest more than once (e.g., during the middle and at the end of the test). If growth is vigorous, grasses may be harvested at a pre-arranged height, e.g., 2 to 6 cm above soil surface during the middle of the 12-week test period. Multiple harvests permit evaluation of both gross plant yield and plant uptake of the test substance with respect to age of the plants (Elseewi et al. 1980; Van Voris et al. 1984, 1985).

5.5 Exposure Techniques

5.5.1 If the primary mode of exposure of the test chemical is anticipated to be by addition of pH adjusted reverse osmosis (RO) water or rainwater containing appropriate concentrations of the test substance, then this procedure should be followed. Again, the following procedure is used unless information is available that suggests some other method should be used or that the chemical is associated with other chemicals or solvents. In no case shall the total aqueous volume of exposure be sufficient to cause microcosm leaching (i.e., > field capacity). Recommended concentration levels are discussed in Sections 5.3 and 5.4. Test substances which are likely to be released into the environment as a liquid or powder, and which can be mixed with water, are applied as a single dose of liquid sufficient in volume to bring the microcosm soil surface horizon to field capacity. The volume

of RO or rainwater required for exposure can be determined on an unplanted microcosm of the same soil type on site; the volume selected should be identical for all microcosms. Carriers other than water are not recommended unless they are likely to be released into the environment in the same effluent stream or accidental spill of the test substance. If a carrier is necessary, then acetone or ethanol should be considered; however, the use of carriers should be avoided unless they are essential to produce a realistic exposure.

5.5.2 Several typical exposure modes are suggested for particular types of test substances if either a hypothetical or real (actual) exposure scenario is not available. If the test substance is likely to be a contaminant of irrigation water, it should be applied every week in proportionate concentrations, such that the total amount applied equals the desired treatment level. If the test substance does not mix with water, it should be applied as evenly as possible to the top of the unplanted microcosm and mixed into the topsoil prior to planting. If the test substance is normally sprayed on growing plants (e.g., pesticide), then the desired amount should be mixed with the volume of water necessary to wet the soil surface and wet the plants to the point where they begin to drip. A chromatography sprayer should be used to spray plants that are past the seedling stage. The recommendations by the test substance's manufacturer for field spraying should be

followed as closely as possible, but the test should be terminated (last harvest) at least eight weeks after the plants are sprayed.

5.6 Waste Disposal

5.6.1 All liquid (leachate) and solid (soils and plant tissues) samples must be retained for proper disposal. All sample collection bottles, collection apparatus, microcosm tubes (Driscopipe®) and sampling tools should be thoroughly cleaned (acid washed) and analyzed for radioactive contamination before they are stored or used on another test system. All samples and the remaining, undisturbed portion of the test system should be disposed of in accordance with EPA and Nuclear Regulatory Commission (NRC) regulations, if radiolabeled compounds were used. Soil leachate and all other aqueous-sample wastes should be treated prior to disposal using one or more of the following techniques:

(a) filtration

(b) activated charcoal filtration

(c) ion exchange.

5.6.2 Soils contaminated with organic residues and/or radiolabeled compounds as well as the Driscopipe®, sample bottles, glassware, gloves, masks, filters, activated charcoal from aqueous cleanup, and any other potentially contaminated equipment must be either certified as uncontaminated or packaged and

disposed of in accordance with existing EPA and NRC guidelines and regulations.

6.0 FATE AND EFFECTS SAMPLING PROCEDURES

Sampling procedures have been divided into two basic categories: ecological effects sampling and test-chemical fate sampling. Ecological effects sampling may include productivity measurements, physical appearance of plants, and nutrient loss or uptake measurements. Test-chemical fate sampling may include leachate, soil, and plant analyses.

6.1 Ecological Effects Sampling

6.1.1 Productivity Measurements

(1) Primary productivity is a commonly measured parameter in terrestrial effects testing. Depending on the plant species, it may be desirable to report total yield or yield by plant part. For example, in the case of grain crops such as soybeans, oats, and wheat, the total biomass yield can be reported in addition to the grain yield. This will allow a relative comparison of total biomass yield with grain yields typically reported for local agriculture. In addition, separate grain samples may be useful for later tissue analyses to determine whether the test chemical was enriched in potentially edible plant parts. For other systems, such as natural grassland microcosms, segregation into plant parts may be unnecessary.

(2) At a minimum, productivity should be reported as oven-dry

weight. Jones and Steyn (1973) recommend 65°C for 24 hours as adequate conditions for drying without unnecessary thermal decomposition of plant material. Information on the chemical's volatility should be evaluated when selecting a drying temperature. It may be desirable in some circumstances to report air-dry productivity or to be able to calculate air-dry yields based on moisture loss after oven-drying. These data could be useful if agricultural crops are the plants used in the microcosm and if it is desirable to compare productivity with yields reported in local agriculture.

(3) The number of harvests will depend on the plants grown. An agricultural crop, alfalfa/timothy for example, may require two or more harvests over the course of the testing period (Malanchuk et al. 1980; Van Voris et al. 1984).

6.1.2 Physical Appearance of Plants

Throughout the test period, it is desirable to record the physical appearance of plants in the terrestrial microcosm. Symptoms of nutrient deficiency or toxicity, pathogenicity, water stress, or test-chemical-induced toxicity should be monitored. These observations may be useful in interpreting the specific ecological effects of a test chemical relative to responses in plants elicited by known environmental toxicants or stresses (Daubenmire 1959). Careful observation on physical appearance in controls vs. treated microcosms may also aid in determining whether abnormal physical appearance is a result of

the test chemical or is a manifestation of microcosm management.

6.1.3 Nutrient Loss Measurements

(1) An important ecological effects sampling procedure is to monitor nutrient losses in leachates (O'Neill et al. 1977; Van Voris et al. 1980; Jackson et al. 1978; Jackson et al. 1979). The rationale for such monitoring is explained in detail in the accompanying support document. One of the desirable attributes of the terrestrial microcosm approach to testing chemicals is the relative ease with which soil leachates can be collected. This offers the potential to construct nutrient budgets for the model ecosystem (Schindler et al. 1980; Gile and Gillett 1979).

(2) The final suite of nutrients monitored in leachates probably will depend on the nature of the test chemical Ausmus et al. 1978; Ausmus et al. 1979; Jackson et al. 1979; Harris et al. 1980). Those nutrients which initially should be considered during the range-finding test include calcium, potassium, nitrate- nitrogen, ortho-phosphate, ammonium-nitrogen, and dissolved organic carbon (DOC). Depending on the results of nutrient losses measured during the range-finding test, a set of nutrients can be selected for monitoring during the definitive test.

(3) Various methods exist to analyze for the nutrients. Standard techniques which have proven useful include atomic

absorption spectrophotometry for Ca and K and analysis using a Technicon Autoanalyzer II for nitrate-nitrogen, ortho-phosphate, DOC, and ammonium-nitrogen (Association of Official Analytical Chemists (AOAC) 1975; American Society for Testing and Materials 1979; EPA 1979). For less rigorous determinations, e.g., during the range-finding test, ion-specific electrodes may be useful for nitrate- and ammonium-nitrogen.

(4) A standard procedure, described below, has proven to be useful in handling leachates. As soon as soil water samples are collected, the sample volume is recorded and the pH determined using a glass electrode. Samples are centrifuged at low speed (e.g. 5000 rpm) to remove large particulates and filtered through a 0.45-micron filter. The sample should be divided into two aliquots prior to storage in the dark at 4°C. Blanks consisting of distilled water and reference standards in instrument calibration quantities should be prepared and stored similarly.

6.2 Test-Chemical Fate Sampling

6.2.1 The fate of the test chemical (see EPA 1982b) will be determined by methods appropriate to the test, including sensitivity factors adequate to verify exposure and distinguish between parent material, transformation products, and naturally occurring materials present in the test system. Usually this

will involve use of a radiolabeled parent compound and subsequent analysis of microcosm components for radioactivity and chemical identity. Methods appropriate to the latter may be adequate for quantification of fate, but usually cannot reveal bound residues in soil or plants and frequently are inadequate for cost-effectively tracing movement and transformation. To the extent that the fate in soil and plants is well enough understood from other experiments and depending on the degree to which the microcosm test is being employed to verify fate and exposure hypotheses, analytical requirements may be reduced (Harvey 1983; Lichtenstein et al. 1972; Lichtenstein et al. 1973; Metcalf et al. 1973; Cole et al. 1976).

6.2.2 Radiolabeling the Parent Compound

The parent compound may be labeled with ^{14}C either in an appropriate aromatic, cyclic carbon group, or linear chain (Lichtenstein et al. 1974). Other labels, including stable isotopes such as ^{15}N may be more useful and informative. In order for the microcosm test to permit an analysis of the fate of the parent compound and/or its metabolites, attention should be paid to known or hypothesized metabolic pathways for test substances. Hence the site and form of label is an integral part of the total test design. The laboratory conducting the test is not required to have the capability for radiolabeling, since this is routinely handled by speciality chemical firms. Sufficient radioactivity must be present in order to detect at

least 1% of the initial parent compound in a typical sample of leachate, soil or plant tissue.

6.2.3 Compartment Analysis for Labeled Compounds

(1) Several compartments of the terrestrial microcosm can be analyzed for radioactivity, which include: samples of soil leachate, plant tissue, including roots and shoots; and soil from three depths. The three soil depths used for radiochemical analyses should be selected based on information on soil sorption of the compound of interest. From previous experience, these depths should be relatively close to the soil surface (1-2 cm) for radiolabeled chemicals that are strongly sorbed to soils; if any isotope appears in the leachate, the depth selection should be lower in the soil profile. Samples will be homogenized and extracted with solvents appropriate for the parent compound. Additional extraction steps may be necessary such as acidification and extraction with non-polar solvents, sohxlet extractions with polar and/or non-polar solvents, alkaline or acid hydrolysis with or without heat, detergent extractions, or protease digestion. The ^{14}C in the soil or plant samples which cannot be extracted will be oxidized and analyzed as $^{14}CO_2$ as described by Lichtenstein et al. (1972) or dissolved in Protosol and Aquasol as described by Cole et al. (1976). The extracts and the oxidized or dissolved samples will be counted by ^{14}C liquid scintillation (Metcalf et al. 1973; Cole et al. 1976).

(2) At the termination of the range-finding test, soil samples will be collected from the top, middle, and bottom of the 60-cm soil cores. If the labeled compounds or its metabolites are not detected by liquid scintillation in the deeper soil samples, then soil samples at the end of the definitive test should be taken closer to the top of the soil column.

6.2.4 Identification of Degradation Products

Liquid scintillation will identify the presence of ^{14}C-labeled compounds in sample extracts, but the identification and quantification of the parent compound or its degradation products will require gas-liquid chromatography (GLC), and thin-layer chromatography (TLC) or high performance liquid chromatography (HPLC) (U. S. EPA 1982b). TLC autoradiography using no-screen X-ray film for chromatographed fractions found to be radioactive by liquid scintillation counting (Cole et al. 1976; Metcalf et al. 1973) is most cost-effective. Whenever possible, the identity of the parent compound and probable degradation products in fractions found to be radioactive by liquid scintillation counting (Cole et al. 1976; Metcalf et al. 1973) will be verified by gas-liquid chromatography methods. Also, the concentration of the parent compound and degradation products should be verified by an alternative chromatographic methods system (e.g., HPLC or GLC) with known standards.

7.0 DATA ANALYSIS

7.1 Ecological Effects Analysis

A combination of statistical analysis procedures are suggested to evaluate the data collected to discern ecological effects. Regression, correlation, and covariance analysis, as well as analysis of variance (ANOVA) procedures described in the following section are suggested as appropriate methods for data analysis. A number of statistical references should be consulted prior to performing the data analysis (Sokal and Rohlf 1981; Snedecor and Cochran 1980; Steel and Torrie 1980). The level of significance for all statistical tests is set at the 5% level ($\alpha = 0.05$) unless otherwise defined. The results of all statistical tests performed on all data must be fully documented for evaluation. In addition, graphs displaying the data and tables containing all raw numbers should be appended to the submittal.

7.1.1 Test Material Effects

Because the mode of exposing the test system can be done without the use of carrier chemicals (see Section 5.2.3 and 5.5.1), there is no need to perform separate analyses on carrier effects as suggested in the marine and freshwater microcosm protocols. This may be mandatory however, if it becomes advisable to use carrier chemicals.

7.1.2 Productivity of Natural or Planted Vegetation

The sum total of both air-dry and oven-dry biomass expressed in g/m^2 collected during and at the end of the definitive test should be evaluated initially by using side-by-side histograms displaying the calculated means, variances, 95% confidence intervals, and 2 Standard Errors for controls and all exposure levels. This will allow early visual evaluation of the effects of the chemical by exposure level and indicate whether logarithmic or some other transformation of the data may be necessary for graphic display and analysis. Analysis of variance (ANOVA) calculations (Sokal and Rohlf 1981; or Steel and Torrie 1980) should be carried out first to test for position effects within the carts and within the environmental area where the test was performed. Position effects may be accounted for in the design of the experiment (e.g., by blocking and any effect of position can then be accounted for in subsequent analyses. If these tests prove to be significant at the 5% level (α = 0.05), then this will need to be accounted for in the remainder of the statistical analyses. Pair-wise comparisons of variables that are measured only once during the 12-week experiment may be necessary (Snedecor and Cochran 1980). In order for ANOVA procedures to be applicable, all experimental microcosms must be assigned to the experimental treatment level by some random process. This may be accomplished through use of a completely randomized experimental design,

randomized block design, Laten-square design or another appropriate experimental design. If not, the results and analysis may be biased, thus jeopardizing the outcome of the experiment. One-way ANOVA procedures should be performed on biomass data in order to determine if an ecological effect resulted from the treatment levels of the parent compound and transformation products. If the ANOVA is significant, then a Least Significant Differences (LSD) Multiple-Range Procedure, such as Duncan's or Dunnet's test methods, should be performed in order to determine which of the treatment means were different from the others. The undosed controls are considered to be one of the treatment levels. Again, the 5% level ($\alpha = 0.05$) should be considered as the level of significance for all tests. All values, whether significant or not, must be reported for each statistical test being performed. Where treatment effects and interactions between and among various factors is important, then a two-way ANOVA or factorial analysis is more appropriate.

Regression/correlation analysis should subsequently be performed on the productivity results. Outlier data, defined as an obvious data recording or reporting error, should be excluded; however, these data and the fact that they have been excluded must be reported. If a substantial number of data points have been declared as outliers, deficiencies in quality control may necessitate rerunning the test. Once outlying

values have been detected and removed from further statistical evaluations, regression models or Probit analysis can be used to estimate the concentration where 50% of the productivity realized in the controls occurred for the test substance (EC_{50}). Ordinary linear least squares regression analysis should initially be performed and the predicted responses in each group should be compared by using Student t-test (one-sided) comparisons. One-sided tests should be used initially because biomass yields are a priori expected to either decrease or increase with increasing exposure level. If it appears that productivity is bimodal when compared to controls then a two-sided Student t-test may be necessary. In addition, it may be necessary to transform the data or fit either a quadratic or cubic least-squares-regression model to the data for this type of response. Position effects should be included in the data. Utilization of computer software packages such as SAS (Statistical Analysis System) or BMDP (Biomedical Computer Program) may prove useful.

7.1.3 Physical Appearance of Plants

Changes in physical appearance in plants in the terrestrial microcosms should be reported for all test units. No statistical evaluations of the effects of the chemical and transformation products on appearance are to be performed unless there is a clearly identifiable pattern of effects. In this case, clearly recognizable patterns of injury to plants may be ranked

in terms of severity. A non-parametric test, such as Kruskal-Wallis test, may be conducted to test for differences in plant injury.

7.1.4 Nutrient Losses

Based on the nutrient or nutrients selected for analysis in the soil leachate (see Section 6.3.1) collected from each microcosm, the total cumulative nutrient loss for each test system for each nutrient should be calculated by (a) multiplying nutrient loss concentration from each collection date times the total volume leached from that microcosm for that collection date, and (b) then adding it to the previous sum of total loss. Plots of the collection date means (±SE) of the cumulative nutrient losses for each treatment level should be developed as a function of days after seeding for the agricultural microcosm or days after exposure for the natural grassland microcosm. Zero loss should be the starting point; if there was no leachate for any microcosm during a particular collection period, the data point should be recorded as zero so that no data are considered missing.

A one-way ANOVA should be performed on the total cumulative nutrient loss data at the end of the experiment, to evaluate the treatment level effects. A Multiple-Range Procedure such as Duncan's should be used in order to determine which specific treatment means were different from each other.

Regression/correlation analysis comparing nutrient losses versus productivity should be performed to determine the relationship between these two independently measured variables. In order to perform this analysis the productivity and cumulative leachate loss measures, must be matched unit for unit. This test should be performed for each nutrient analyzed versus productivity.

7.2 Chemical Fate Analysis

At the end of the experiment, the budget or distribution of the parent compound and transformation products is calculated for each exposure (concentration) level. This entails determining the amount added and the subsequent distribution of the radioactivity of the parent compound and transformation products through chemical analysis in each of the primary compartments, soil, H_2O, plant tissue and air of the test system as well as in the soil leachate. Fate analysis should result in distribution values for above-ground plant tissues, plant roots, each soil depth (see Section 6.2.3), and loss to soil leachate. Gaseous loss can be estimated. This is followed by performing statistical analyses for each exposure level on any differences in distribution of the compound throughout the test system. Multi-compartmental modeling and multivariable analyses such as multivariate analysis of variance may also prove useful in assessing the fate of parent compounds and transformation products.

7.2.1 Radioactivity Budgets

The calculation of a complete budget of all radioactivity is required to be submitted with the results of this test. The budget must show the percent of the compound that was tagged and the location of the tag. The label may be ^{14}C, stable ^{15}N, or another suitable label and should be located in a portion of the molecule expected to persist and/or have biological activity.

(1) Total Radioactivity Added

The total radioactivity added per test unit is based on the decay rate of the radioactive tag, the total amount of radioactive tag added to the compound when initially formulated, the length of time between formulation and test unit exposure (radioactive decay), and the particular exposure level of the test unit.

(2) Total Radioactivity Removed

The total radioactivity removed from the microcosm is based on the concentration of the radiolabel in (a) the soil leachate concentration times the volume of soil leachate lost per collection date, (b) the calculated gas phase losses of the compound, and (c) the type of radiolabel and the rate of radioactive decay of that label over the length of the experiment.

(3) Total Radioactivity Remaining

The total radioactivity remaining in the microcosm is based on

radiolabel analysis of each of the primary compartments (a) above-ground plant tissues, (b) plant roots (i.e., cleaned of soil particles) and (c) the distribution of the label through the different soil depths. The soil depths to be analyzed will be based on a range-finding evaluation as suggested in section 6.2.3.

7.3 Fate of Parent Compound and Transformation Products

Calculations should be made for each exposure (concentration) level relative to the percent distribution of the test substance for (a) above-ground plant tissues, (b) below-ground plant tissues, (c) each depth through the soil profile, (d) losses to soil leachate, and (e) calculated total vaporization or gaseous losses. In addition, an analysis of the time to reach steady-state loss of the chemical compound in the soil leachate and the time to initiate compound leaching from each exposure level should be calculated. For those exposure levels where the time to reach steady state loss in the soil leachate is greater than the length of the experimental period (12 weeks) then an asymptotic regression model should be used to determine the time necessary to reach steady state (Snedecor and Cochran 1980).

The calculations for the final distribution of the test material and transformation products are based on the measured radioactivity in that compartment on a per gram basis times the

total weight or volume of material in that compartment, expressed on a dry weight basis where appropriate. All calculations, are subsequently corrected for the radioactive decay occurring since the beginning of the experiment. The quantities of compound are to be expressed as a percent of the original compound added to the test system. An ANOVA test should be performed on the calculated mean percentage remaining for each of the four main compartments for all exposure concentrations. It may be necessary to transform the percentage data prior to ANOVA in order to satisfy the assumptions of the analysis. In addition, regression/correlation analyses should be performed comparing above-ground productivity versus concentration of parent compound and transformation products in above-ground plant tissues, and cumulative nutrient losses for each exposure concentration versus parent compound and transformation products in the soil leachate.

7.4 Bioconcentration and Enrichment

(1) Calculate the concentration of the radioactivity in the above-ground plant tissues and in the top 15 cm of soil on a concentration per unit dry weight basis. The ratio of the plant tissue concentration to soil concentration is then defined as the Bioconcentration Factor (BF). Side-by-side histograms displaying the BF ratios should then be compared for statistical differences.

(2) Enrichment ratios (ER) should be calculated for the higher exposure levels by dividing the activity expressed on a dry weight basis in a higher exposure level by the lowest exposure level in which a significant (i.e., detectable) concentration could be analytically determined. The enrichment ratio is then regressed against exposure concentration to determine the linearity of the uptake of the chemical as measured through the radiolabeled portion of the chemical. If it appears that an ordinary least squares regression analysis is not the appropriate function defining the enrichment of the chemical then an attempt to fit a quadratic or cubic equation should be made. The above guidelines for calculating BFs and ERs assume that no wet analytical chemistry has been performed to separate the parent compounds from the transformation products in each compartment. If this has been done, then individual BFs and ERs should be calculated as outlined above.

8.0 TEST PROTOCOL BIBLIOGRAPHY

Association of Official Analytical Chemists. 1975. Official Methods of Analysis of the Association of Official Analytical Chemists. 12th Edition. Washington, D.C., 1094 pp.

American Society for Testing and Materials. 1979. Annual book of ASTM Standards. Part 31, Water. American Society for Testing and Materials, Philadelphia, PA. 128 pp.

Ausmus BS, Dodson, GF, & Jackson DR. 1978. Behavior of heavy metals in forest microcosms: III. Effects on litter-soil carbon metabolism. Water Air Soil Pollut. 10:19-29.

Ausmus BS, Kimbrough S, Jackson DR, & Lindberg S. 1979. The behavior of hexachlorobenzene in pine forest microcosms: transport and effects on soil processes. Environ. Pollut. 20:103-111.

Black, CA (ed). 1965a. Methods of Soil Analysis I. Physical and Minerological Properties. American Society of Agronomy, Madison, WI. 770 pp.

Black, CA (ed). 1965b. Methods of Soil Analysis II. Chemical and Microbiological Properties. American Society of Agronomy, Madison, WI. 802 pp.

Cairns J, Jr. 1981. Testing for effects of chemicals on ecosystems. National Academy of Science, Washington, D.C. 103 pp.

Cairns J, Jr. ed. 1985. Multispecies Toxicity Testing. Pergamon Press, New York. 253 pp.

Cole LK, Sanborn JR, & Metcalf RL. 1976. Inhibition of corn growth by aldrin and the insecticide's fate in the soil, air, crop, and wildlife of a terrestrial model ecosystem. Environ. Entomol. 5:583-589.

Daubenmire, R. 1959. A canopy cover method of vegetation analysis. N.W. Science 33: 43-64.

Elseewi AA, Straughan IR, & Page AL. 1980. Sequential cropping of fly ash-amended soils: Effects on soil chemical properties and yield and elemental composition of plants. Sci. Tot. Environ. 15:247-259.

Environmental Protection Agency. 1979. Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples. Health Effects Research Laboratory. Research Triangle Park, N.C.

Environmental Protection Agency. 1982a. Environmental Effects Test Guidelines. Washington, DC. Office of Pesticides and Toxic Substances, U.S. EPA. EPA 560/6-82-002. Available from NTIS, Springfield, VA PB82-232992.

Environmental Protection Agency. 1982b. Chemical Fate Test Guidelines. Washington, DC. Office of Pesticides and Toxic Substances, U.S. EPA. EPA 560/6-82-003. Available from NTIS, Springfield, VA PB82-233008.

Gile JD, & Gillett JW. 1979. Fate of selected fungicides in a terrestrial laboratory ecosystem. J. Agric. Food Chem. 27:1159-1164.

Harris WF, Ausmus BS, Eddlemon GK, Giddings JM, Jackson DR, Luxmore RJ, O'Neill EG, O'Neill RV, Ross-Todd M, and Van Voris P. 1980. Microcosms as potential screening tools for evaluating transport and effects of toxic substances. ORNL/EPA-4, EPA-600/3-80-042. Environ. Sci. Div. Publ. No. 1506, Oak Ridge, TN. 453 pp.

Harvey J, Jr. 1983. A simple method of evaluating soil breakdown of ^{14}C pesticides under field conditions. Residue Rev. 85:149-158.

Jackson DR, Selvidge WJ, & Ausmus BS. 1978. Behavior of heavy metals in forest microcosms: II. Effects on nutrient cycling processes. Water Air Soil Pollut. 10:13-18.

Jackson DR, Ausmus BS, & Levin M. 1979. Effects of arsenic on nutrient dynamics of grassland microcosms and field plots. Water Air Soil Pollut. 11:3-12.

Jones JB, & Steyn WJA. 1973. Sampling, handling, and analyzing plant tissue samples, pp. 249-270. In: LM Walsh and JD Beaton eds. Soil Testing and Plant Analysis. Soil Science Society of America, Inc., Madison, WI.

Laskowski DA, Goring CAI, McCall PJ, & Swann RL. 1982. Terrestrial environment, pp. 198-240. In: RA Conway ed. Environmental Risk Analysis for Chemicals. Van Nostrand Reinhold Company, New York, NY.

Lichtenstein EP, Schulz KR, & Fuhremann TW. 1972. Movement and fate of dyfonate in soils under leaching and nonleaching conditions. J. Agr. Food Chem. 20:831-838.

Lichtenstein EP, Fuhremann TW, Schulz KR, Liang TT. 1973. Effects of field application methods on the persistence and metabolism of phorate in soils and its translocation into crops. J. Econ. Entomol. 66:863-866.

Lichtenstein EP, Fuhremann TW, & Schulz KR. 1974. Translocation and metabolism of (^{14}C) phorate as affected by percolating water in a model soil-plant ecosystem. J. Agr. Food Chem. 22:991-996.

Malanchuk JL, Mueller CA, & Pomerantz SM. 1980. Microcosm evaluation of the agricultural potential of fly-ash-amended soils, pp. 1034-1049. In: JP Giesy, Jr. ed. Microcosms in Ecological Research, CONF-781101. U.S. Department of Energy, Technical Information Center.

Metcalf RL, Kapoor IP, Lu P, Schuth CK, & Sherman P. 1973. Model ecosystem studies of the environmental fate of six organochlorine pesticides. Environ. Hlth. Perspect. 4:35-44.

O'Neill RV, Ausmus BS, Jackson DR, Van Hook RI, Van Voris P, Washburne C, & Watson AP. 1977. Monitoring terrestrial ecosystems by analysis of nutrient export. Water Air Soil Pollut. 8:271-277.

Rand GM. 1980. Detection:bioassay. Pages 390-403. In: Guthrie FE & Perry JJ., eds. Introduction to Environmental Toxicology. Elsevier North Holland, Inc., N.Y. 484 pp.

Schindler JE, Waide JB, Waldron MC, Hains JJ, Schreiner SP, Freeman ML, Beng SL, Pettigrew DR, Schissel LA, & Clark PJ. 1980. A microcosm approach to the study of biogeochemical systems. I Theoretical rationale, pp. 192-203. In: JP Giesy, Jr. ed. Microcosms in Ecological Research, CONF-781101. U.S. Department of Energy, Technical Information Center.

Snedecor GW & Cochran WG. 1980. Statistical Methods. 7th Edition. Iowa State University Press, Ames, Iowa.

Sokal RR & Rohlf FJ. 1981. Biometry: The principles and practice of statistics in biological research 2nd Edition. W. H. Freeman and Company, San Francisco, CA.

Steele RDG & Torrie BH. 1980. Principals and Procedures of Statistics 2nd Edition. McGraw-Hill Book Co., N.Y. 481 pp.

Swann RL, Laskowski DA, McCall PJ, Vander Kuy K, & Dishburger HJ. 1983. A rapid method for the estimation of the environmental parameters octanol/water partition coefficient, soil sorption constant, water to air ratio, and water solubility. Residue Reviews. 85:17-28.

Tolle DA, Arthur MF, Van Voris P, Feder PI, Matthews MC, Chesson J, Larson ME, & Zwick TC. 1982. Evaluation of terrestrial microcosms for environmental studies of utility wastes. Third Ann. Prog. Rep. on RP 1224-5 to Electric Power Research Institute, Palo Alto, CA.

Tolle DA, Arthur MF & Van Voris P. 1983. Microcosm/field comparison of trace element uptake in crops grown in fly ash-amended soil. Sci. Tot. Environ. 31: 243-261.

Van Voris P, O'Neill RV, Emanuel WR, & Shugart Jr, HH. 1980. Functional complexity and ecosystem stability. Ecology 61(6):1352-1360.

Van Voris P, Arthur MF, & Tolle DA. 1982. Evaluation of terrestrial microcosms for assessing ecological effects of utility wastes. EPRI Publication No. EA-2364. Electric Power Research Institute, Palo Alto, CA. pp. 178.

Van Voris P, Arthur MF, Tolle DA, Morris JP, & Larson M. 1983. Use of microcosms for monitoring nutrient cycling processes in agroecosystems. p. 171-182. In: R. Lowrance, R. Todd, L. Asmussen, and R. Leonard (eds.) Nutrient Cycling in Agricultural Ecosystems. University of Ga. College of Agric. Exp. Station, Spec. Pub. 23. Tifton, GA.

Van Voris P, Tolle DA, Arthur MF, Chesson J, & Zwick TC. 1984. Development and validation of terrestrial microcosm test system for assessing ecological effects of utility wastes. Final Project Report RP1224-5. EPRI Publication No. EA-3672. Electric Power Research Institute, Palo Alto, CA. pp. 185.

Van Voris P, Tolle DA, Arthur MF, & Chesson J. 1985. Terrestrial Microcosms: validation, applications, and cost-benefit analysis. pp 117-142 In: J Cairns, Jr. ed. Multispecies Toxicity Testing. Pergamon Press, New York.

Zwick TC, Arthur MF, Tolle DA, & Van Voris P. 1984. A unique laboratory method for evaluating agro-ecosystem effects of an industrial waste product. Plant Soil. 77: 395-399.

SUPPORT DOCUMENT

CONGRESSIONAL MANDATE

The U.S. Environmental Protection Agency (EPA) is responsible for developing and implementing an efficient and effective scheme for testing environmental toxicity of existing and new chemicals. The EPA was given this responsibility under the Toxic Substance Control Act (TSCA) passed by Congress in 1976 (P.L. 94-469, U.S.C., Section 2601-2629). The Act specifically requires that EPA develop and publish test standards that yield data on a chemical's potential to affect human health and the environment. With the publication of the preceding test guideline and this support document for performing the "Experimental Terrestrial Soil-Core Microcosm Test Protocol," EPA is meeting one of its specific responsibilities as set forth in TSCA.

This document provides a general rationale for the approach and materials suggested for use in the soil-core microcosm. It also adds more detail on the use of this experimental test system to evaluate the environmental fate, ecological effects and environmental transport of chemicals. The test results from this and other tests can then submitted by industry for review by EPA Office of Pesticides and Toxic Substances (OPTS).

RATIONALE FOR MICROCOSM USE

The fate and ecological effects of a chemical are influenced by the chemical's characteristics (e.g., physical, chemical and bioactivity), by environmental characteristics (e.g., soil type, and its physical and chemical properties), and by environmental conditions (e.g., wet vs. dry, and cool vs. warm climate). To supply this information in a uniform and consistent manner, tests like those published in EPA (1982a and b) or presented here must be performed on the chemical to evaluate its environmental toxicity and fate at all four levels of ecological organization (i.e., organism- population-community-ecosystem).

It is generally recognized that current test guidelines (EPA 1982a and b) deal with only the first two of four levels of ecological organization. Moreover, these protocols deal exclusively with physiologic and abundance attributes of organism and population levels of organization. Single-species bioassay systems performed under acute-exposure regimes are designed to yield reproducible, quick, cost-effective, and unequivocal answers that are applicable to the organism and population levels of organization. However, relying exclusively on single-species bioassays, coupled with the characteristics of the chemical, increases the likelihood of

Type II error. Both O'Neill and Waide (1981), and Cairns (1981) suggest that ecosystems can and have played a significant role in potentiating a chemical's toxicity to the extent that current bioassay protocols are unable to reduce the probability of false negatives. (Reliance solely on current protocols increases the likelihood of serious, potentially irreversible environmental damage.) Therefore, it is essential that effects likely to occur at the community and ecosystem levels be evaluated directly. This does not exclude or diminish the utility of the single-species test methods, but it does suggest that any ecotoxicology test scheme that is imposed or suggested for use by federal regulations needs to encompass the potential impact of a chemical substance on all four levels of ecological organization. A comprehensive test scheme must be able to accurately determine or predict impacts that might occur in each of the primary environments (marine, freshwater and terrestrial), at each level of ecological organization. We are not suggesting that tests must be performed on each component of the ecosystem, but that whatever tests are performed be designed to protect the most sensitive or vulnerable portion of the system as well as being able to be used as predictive tools for other portions of the ecosystem.

In the past, the primary stumbling blocks to incorporating test protocols that would allow for ecosystem-level evaluations have

been lack of one or more of the following items: 1) validated test protocols, 2) replicable test systems, 3) reproducible test results, and 4) cost-effective techniques. Some of the test systems that have been suggested to date either have bewildering complexity, lack any resemblance to an ecosystem, or are too generic to be of any direct use in the assessment process (Metcalf et al. 1979; Gile and Gillett 1977; Gile et al. 1979; Lichtenstein 1977). The terrestrial soil-core microcosm test system described in this test guideline overcomes many of the faults of the previous multi-species test systems and can be used to address several levels of ecological complexity without sacrificing accuracy, replicability, reproducibility, and cost-effectiveness. Note, however, that the microcosm test system will not duplicate all aspects of the ecosystem that are being investigated, and that any criteria used to evaluate the validity of the microcosm should be equally applied to all environmental test procedures. As with any test system, there are limitations resulting from both scale and sampling.

Limitations imposed by sampling can result from the sample itself and from the method of sampling. First, the intact/extracted microcosm is a subset or sample of a natural ecosystem and, therefore, cannot be expected to capture more than the inherent heterogeneity of the system from which it is

taken. This makes a microcosm a sample (i.e., model ecosystem), not an entire ecosystem. When used as an ecosystem analog, the microcosm must capture the primary functional and dynamic processes characteristic of that system and must contain the dominant biotic components as well as the abiotic sources and sinks.

The method of sampling for analysis of transport, fate, and effects may also limit types of questions that can be addressed with microcosms. Acts of destructive sampling can be significant perturbations in themselves and must be taken into consideration when interpreting the results. Thus, it is critical that nondestructive sampling techniques be considered when designing a microcosm test. In the portion of the Test Protocol entitled: "Terrestrial Microcosm Extraction and Maintenance" (see Section 4.0), special effort was made to avoid destructive sampling of the system except where necessary and appropriate for agricultural ecosystems. An alternative experimental design, that allows for destructive sampling, is to sacrifice periodically one of the replicate microcosms from each treatment level throughout the experiment. Depending on the length of the experiment and the desired destructive sampling rate, this may require that a large number of test systems be extracted at the beginning of the experiment.

Microcosms are limited in scale both temporally and spatially, which in turn limit the size of organisms that can be included in the test system. Elaborate efforts have been made to include large grazers or carnivores in some microcosm test systems (Giesy 1980). A few years ago, large systems were considered better than small systems because they contained small rodents or birds (Cole and Metcalf 1977; Gillett and Witt 1977) or, in the case of the aquatic microcosm, herbivorous or carnivorous fish (Maki 1980; Bowling et al. 1980). The size of the microcosm controls the nature of the questions that can be scientifically and logically addressed. The size of the soil-core microcosm (17-cm x 60-cm) precludes trees or corn, or other large crops. Coupling a microcosm with a single-species toxicity test, such as an avian feeding study, and/or using the chemically contaminated water, vegetation or soil from the microcosm for follow-up exposure or feeding studies, appears to be the more appropriate route to follow. Although most microcosms cannot accommodate large or even medium-size organisms for long periods of time, many functionally dependent phenomena that apply to these organisms can be adequately addressed in the microcosm if the scaling factor is clearly defined and taken into account at the start of the experiment. Examples of scale adjustments include changing surface to volume ratios and selecting crops whose reproductive time is well within the experiment duration.

The type of vegetation or crop that can be grown in the microcosm is dependent on both diameter and depth of the soil core. Large crops such as corn or sorghum are not candidates for this particular agricultural test system because only one plant could be grown at a time, thus limiting the sampling that can be performed. Tests suggest that a mixture of grasses and broad leaves such as legumes should be included in the agricultural microcosm. Two or three species of grasses or legumes should be selected that represent the area or region where the chemical might be accidentally released or applied to crops or soil. The species chosen must be compatible from a competition standpoint and must be able to grow to maturity in the small surface area (240.5 cm^2) of the microcosm. A grain crop could be selected to evaluate the potential for uptake of the parent compound or its transformation product into a grain grown for human consumption.

RATIONALE FOR MICROCOSM DESIGN

The term microcosm, as defined here, is a confined portion of a natural terrestrial ecosystem that is subject to laboratory controls and can be used to investigate ecological processes and evaluate the environmental toxicity of a chemical (Gillett and Witt 1977; Draggan and Van Voris 1978; Van Voris *et al.* 1980). The ability to manipulate the microcosm's environment

(i.e., temperature, water, and light) is a requirement and results, in part, from the reduction in size. The microcosm must capture and incorporate the primary ecological processes (e.g., photoautotrophy, if present, and nutrient cycling) and allow for population fluctuations and community interactions. Both biotic as well as abiotic components must be included in the structure of the test system, because functioning and persistence of the biotic component is dependent on the abiotic sink for nutrient regeneration, organic complexation, and temporary isolation of internally generated wastes.

Microcosms in the past have been either fabricated from mixed cultures of organisms and artificial substrates (Ausmus et al. 1977; Harris et al. 1980; Cole and Metcalf 1977; Metcalf et al. (1979) or extracted as intact portions of the ecosystems they represent (Van Voris et al. 1980, 1982; Jackson et al. 1978; Harris et al. 1980). Intact microcosms, like the one presented in this test protocol, are believed to be an improvement over other test systems because they preserve to a greater extent system-level interactions such as nutrient cycling processes and plant/microbial interaction, and maintain microsite chemistry of the soil. Failure to incorporate intact undisturbed soil or sediment in a microcosm is the single most common design flaw. Failure to include undisturbed soil or sediment as it exist in the environment deemphasizes the

stratification of biological processes within the ecosystem, may reduce the overall buffering capacity of the system, may eliminate critical chemical activation pathways, and may remove a major sink for chemical contaminants.

Additional support information concerning design rational for this particular test system, and about the utility of terrestrial microcosms in general can be found in journal publications and EPA and EPRI project reports listed at the end of the Test Protocol and this support document. For further details on rationale see:

(1) Gillett and Witt (1977), a National Science Foundation and EPA workshop on Terrestrial Microcosms and Environmental Chemistry

(2) Van Voris et al. (1984), 1984-EPRI Final Project RP1224-5 Report. EPRI Publication No. EA-3672.

(3) Hammons (1981), an Oak Ridge National Laboratory - EPA document on "Methods for Ecological Toxicology: A Critical Review of Laboratory Multispecies Tests"

(4) Harris et al. (1980), an Oak Ridge National Laboratory - EPA document detailing three years of research on both terrestrial and freshwater microcosms

(5) Levin et al. (1982), "New Perspectives in Ecotoxicology"

(6) Cairns (1981), "Testing for Effects of Chemicals on Ecosystem"

(7) Cairns (1985), "Multispecies Toxicity Testing", Pergamon Press, New York. 253 pp.

A microcosm attempts to duplicate the biotic and abiotic conditions of a natural terrestrial ecosystem (Van Voris et al. 1980, 1982). Soil core diameter and depth are somewhat arbitrarily selected. Studies suggest however, that a core 17-cm in diameter is the minimum size capable of sustaining healthy vegetative growth (Harris et al. 1980; Hammons 1981; Van Voris et al. 1984). The microcosm depth is most likely dependent on soil type, but should be deep enough to prevent the system from becoming root bound. Another advantage of the 17-cm-diameter core is that it fits inside the next-to-largest commercially available Buchner funnel, making the materials more cost-effective. Conditions in the natural ecosystem are simulated where appropriate in the microcosms environment to make the test system a more useful tool for predicting ecological fate and effects of chemicals. Simulation involves selecting a relatively undisturbed soil column, insulating the soil column, and controlling the photoperiod used during the test. These factors are all dependent on the conditions of the local environment and emphasizes the use of these test systems for site-specific or regional risk assessments.

RATIONALE FOR MICROCOSM MATERIALS

Materials to house the microcosm were selected for their chemical inertness, cost, reuse, and simplicity in use (Van Voris et al. 1982; Tolle et al. 1982). The three basic materials used for a single microcosm are the 60-cm-long Driscopipe® tube (17.5 cm diameter), a 186-mm-diameter porcelain Buchner funnel, and a thin layer of glass wool (Zwick et al. 1984) (see Figure 1 in Test Protocol). These materials are chemically inert. The Driscopipe® is an ultra-high molecular weight, high density, non-plasticized polyethylene pipe that is highly resistant to acids, bases, and biological degradation.

The combined cost of the three microcosm materials (1985 dollars) is approximately $62.00 per microcosm when purchased in sufficient quantities (60 units) to conduct both a range-finding and definitive test. Microcosm carts cost about $54 each for lumber, wheels, and Styrofoam® insulation. If each cart contains six microcosms, this adds about $9 to the cost of each microcosm. Thus the total cost of materials is approximately $71 per microcosm. Labor costs for core extraction, cart construction and experimental unit setup are not included in this estimate (Van Voris et al. 1985).

Compared to other intact terrestrial microcosms (e.g., Jackson et al. 1978; Van Voris et al. 1980; Gile and Gillett 1977), the system described in this document is simple to construct and the encasement materials can be easily cleaned for reuse with another test chemical. Complex terrestrial microcosm chambers, such as those described by Nash and Beall (1977), Gile and Gillett (1977), and Van Voris et al. (1980) require more complicated procedures and have much higher costs for materials and setup. With the combined cost of materials and labor plus typical overheads to setup (but not perform) the test for one range-finding and one definitive test are estimated to be in the neighborhood of $6,500 to $7,900 (1985 dollars).

RATIONALE FOR TEST PROCEDURES

The extraction, insulation, and sampling of the microcosms have been refined over a period of five years and are supported in Van Voris et al. (1982, 1984 and 1985), Zwick et al. (1984) and Tolle et al. (1982). Modifications to the procedures may be necessary depending on vegetative cover or crops grown, soil type and porosity, and local environmental conditions.

Microcosm Insulation

Soil temperatures at different depths in a microcosm located in an insulated cart have been shown to closely approximate soil

temperatures in a field plot taken at the same depths over a growing season (Van Voris et al. 1984). Therefore, it is important to surround microcosms in carts with Styrofoam insulation in order to maintain a near normal soil temperature profile.

Leachate Collection

The most suitable frequency for leachate water collection has been shown to be about every two to three weeks (Van Voris et al. 1982; Tolle et al. 1982). Approximately 1,200 ml or more of Reverse Osmosis (RO) water must be added to generate leachate from the microcosm. More frequent leaching could cause over watering and lead to root rot, or cause soil conditions to become anaerobic. Frequent leaching can also cause an unnaturally high water regime in microcosms compared to the typical rainfall experienced in the local area. Thus, care needs to be used when establishing a leachate collection plan for the test systems.

Vegetation Sampling

Sampling of vegetation should be performed at the end of the definitive test unless the test is being performed over a longer period, or a crop that matures in a relatively short period is used. Shoots should be clipped approximately 2.5 cm above the soil surface for all exposure levels, and plant

tissues should be separated into dominant parts (e.g., shoot, leaves, flower, and/or seed) where appropriate. Vegetation in the natural grassland microcosm should be clipped again at a uniform height above the soil surface (e.g., 2.5 cm) and, if appropriate, separated into species and plant parts. At minimum, monocotyledons and dicotyledons should be separated for both productivity measurements and for radiochemical assay.

Microbial and Invertebrate Community Sampling

The experimental design and ecological effects sampling parameters described in the protocol do not suggest sampling for either soil microbiological or soil invertebrate effects; however, both are possible. At the end of the experiment, soil samples can be taken from each replicate from each exposure level for sample preparation. For the microbial community, which plays a critical role in nutrient cycling, a soil respiration test can be used to evaluate the production of carbon dioxide-carbon (CO_2) or the uptake of oxygen (O_2). The heterotrophic microbial community experiments should be performed in closed containers amended with a supplemental carbon source, preferably an environmentally relevant organic substrate (Singh and Gupta 1977; Stotzky 1965; Arthur et al. 1984). For soil invertebrates, which play a critical role in nutrient cycling, either a Tulgren or Berlese Funnel extraction procedure can be used for subsequent evaluation of species

composition and relative abundance (Van Voris et al. 1982). Other methods can be used for both soil microbial activity and soil invertebrate sampling; however, full and complete support for the techniques and analysis of data must accompany any report. For alternate approaches see:

(1) Parkinson et al. (1971), "Methods for Studying the Ecology of Soil Micro-organisms"

(2) Phillipson (1971), "Methods of Study in Quantitative Soil Ecology: Population, Production and Energy Flow"

(3) Page et al. (1982), "Methods of Soil Analysis, Part 2; Chemical and Microbiological Properties."

Chemical Fate Sampling

The objective of evaluating chemical fate within the terrestrial soil-core microcosm is to determine the potential routes of exposure to humans and wild and domestic animals. This requires that the total materials budget be calculated from radiolabeled chemical assay data. In addition, bioaccumulation factor (BF) into plant tissues, particularly those plants that may be used for animal feed or human consumption, must be submitted as part of the test results.

The BF concentration information will be compiled for the chemical and compared with mammalian toxicity information to determine the potential for (1) direct toxicity, (2) indirect toxification due to food-chain transport, and (3) long-term

exposures that might lead to carcinogenic or teratogenic responses. Thus, information on bioaccumulation/concentration factor is essential to the risk assessment process.

The use of radiolabeled compounds is recommended in the Test Protocol. Radiolabeled chemical assays by themselves do not allow separation of parent and transformation products. This may result in a conservative assessment, from EPA's viewpoint, about the parent compound, because data is not available to include an assessment of innocuous or even potentially more toxic transformation products. Therefore, if technically skilled scientist and support staff are available along with financial support and the proper equipment (Gas Chromatograph-Mass Spectrometer, or High Performance Liquid Chromatograph) then separation of the concentrations of parent and transformation products within the plant tissue and leachate is recommended.

When calculating a total materials budget for the test system, estimates should be made of the fractions lost through gas transport (volatilization) or decomposition. That fraction, not accounted for by examining the mean (±2 SD), would be the best estimate of material lost. A portion of this may be the result of heterogeneous distribution of the compound. In certain cases the upper boundary of this number may exceed the total radiolabeled compound added. In this case, the upper

boundary of the amount of the compound lost through gas phase transport from the system, or otherwise unaccounted for, would be equal to the lower number (mean - 2 SD) and the lowest loss would be equal to 0 percent.

Cleanup of Experimental Materials

Once the experiment is over, all wastes should be disposed of in accordance with EPA and Nuclear Regulatory Commission (NRC) regulations. All materials used in the experiment should be washed with a laboratory detergent, then acid washed and checked for residual radioactivity. After the materials have been certified clean by a radiation safety officer, then the equipment may be used for another soil-core microcosm test. All solutions that have been used to clean the Driscopipe®, Buchner funnels, and laboratory glassware should be treated as contaminated and disposed of accordingly, unless radioassay and chemistry data indicate otherwise.

DATA ANALYSIS

The data analysis section is considered to be straightforward. The statistics references cited in the text should be more than adequate to support the use of the suggested procedures.

LIMITATIONS IN THE USE OF THE TEST PROTOCOL

As discussed previously, scale and sampling limit significantly the use of the microcosm test. In addition, the test should not be performed with compounds that have boiling points less than 80°C or a vapor pressure that is greater than 25 mm Hg at 25°C. These compounds are not suited for evaluation within this type of experimental system. For compounds which might fall into this class, Van Voris *et al.* (1980) offer an alternative experimental design that may be more appropriate.

REFERENCES FOR SUPPORT DOCUMENT

Arthur MF, Zwick TC, Tolle DA, & Van Voris P. 1984. Effects of Fly Ash on Microbial CO2 Evolution from an Agricultural Soil. Water Air & Soil Poll. 22: 209-216.

Ausmus, BS, Jackson DR, & Van Voris P. 1977. The Accuracy of Screening Techniques. 123-130. In: JM Witt and JW Gillett eds. Terrestrial Microcosms and Environmental Chemistry. National Science Foundation, Division of Problem-Focused Research Applications, Washington, DC.

Bowling, JW, Giesy JP, Kania HJ & Knight RL. 1980. Large-scale microcosms for assessing fates and effects of trace contaminants. 224-247. In: JP Giesy, ed. Microcosms in ecological research. CONF-781101. Technical Information Center, U.S. Department of Energy, Washington, DC. 1,110 pp.

Cairns J Jr. 1981. Testing for Affects of Chemicals on Ecosystems. National Academy of Science, Washington D.C. 103 pp.

Cairns J Jr. ed. 1985. Multispecies Toxicity Testing. Pergamon Press, New York. 253 pp.

Cole, LK, & Metcalf RL. 1977. Predictive environmental toxicology of pesticides in the air, soil, water and biota of terrestrial model ecosystems. 57-74. In: JM Witt and JW Gillett eds. Terrestrial Microcosms and Environmental Chemistry. National Science Foundation, Division of Problem-Focused Research Applications, Washington, DC.

Draggan S & Van Voris P. 1978. The role of microcosms in ecological research. Intern. J. Environ. Studies. 10:1-2.

Environmental Protection Agency. 1982a. Environmental Effects Test Guidelines. Washington, DC. Office of Pesticides and Toxic Substances, U.S.EPA. EPA 560/6-82-002. Available from NTIS, Springfield, VA PB82-232992.

Environmental Protection Agency. 1982b. Chemical Fate Test Guidelines. Washington, DC. Office of Pesticides and Toxic Substances, U.S.EPA. EPA 560/6-82-003. Available from NTIS, Springfield, VA PB82-233008.

Giesy, JP. ed. 1980. Microcosms in ecological research. CONF-781101. Technical Information Center, U.S. Department of Energy, Washington, DC. 1,110 pp.

Gile JD, & Gillett JW. 1977. Terrestrial Microcosm Chamber Evaluations of Substitute Chemicals. 75-85. In: JM Witt and JW Gillett eds. Terrestrial Microcosms and Environmental Chemistry. National Science Foundation, Division of Problem-Focused Research Applications. Washington, DC.

Gile JD, Collins JC & Gillett JW. 1979. The soil core microcosm - a potential screening tool. EPA-600/3-79-089. August 1979. U.S. EPA Corvallis Environmental Research Laboratory, Corvallis, Oregon. 41 pp.

Gillett JW, & Witt JM, eds. 1977. Terrestrial microcosms. The proceedings of the workshop on terrestrial microcosms. Symposium on terrestrial microcosms and environmental chemistry. National Science Foundation, Division of Problem-Focused Research Applications, Washington, D.C. 53 pp.

Harris WF, Ausmus BS, Eddlemon GK, Giddings JM, Jackson DR, Luxmore RG, O'Neill EG, O'Neill RV, Ross-Todd M, & Van Voris P. 1980. Microcosms as potential screening tools for evaluating transport and effects of toxic substances. ORNL/EPA-4, EPA-600/3-80-042. Environ. Sci. Div. Publ. No. 1506, Oak Ridge, TN. 453 pp.

Hammons A, ed. 1981. Methods for ecological toxicology: A critical review of laboratory multispecies tests. ORNL-5708, EPA-560/11-80-026. Ann Arbor Science Publishers Inc., Ann Arbor, MI. 305 pp.

Jackson DR, Selvidge WJ, & Ausmus BS. 1978. Behavior of heavy metals in forest microcosms: I. Transport and distribution among components. Water Air Soil Pollut. 10:3-11.

Levin SA, Kimball SK, Kimball K, & McDowell W. 1982. New perspectives in ecotoxicology. ERC Report No. 14. Ecosystems Research Center, Cornell University, Ithaca, NY. 134 pp.

Lichtenstein EP 1977. Fate of pesticides in a soil-plant microcosm. 95-101. In: J.M. Witt and J.W. Gillett eds. Terrestrial Microcosms and Environmental Chemistry. National Science Foundation, Division of Problem-Focused Research Applications, Washington, DC.

Maki, AW. 1980. Evaluation of toxicant effects on structure and function of model stream communities: correlation with natural stream effects. 583-609. In: JP Giesy, ed. Microcosms in ecological research. CONF-781101. Technical Information Center, U.S. Department of Energy, Washington, DC. 1,110 pp.

Metcalf RL, Cole LK, Wood SG, Mandel DJ, & Milbrath ML. 1979. Design and evaluation of a terrestrial model ecosystem for evaluation of substitute pesticide chemicals. EPA-600/3-79-004. U.S. EPA Corvallis Environmental Research Laboratory, Corvallis, Oregon. 298 pp.

Nash RG & Beall ML, Jr. 1977. A microagroecosystem to monitor the environmental fate of pesticides. 86-94. In: JM Witt and JW Gillett eds. Terrestrial Microcosms and Environmental Chemistry. National Science Foundation, Division of Problem-Focused Research Applications, Washington, DC.

O'Neill RV & Waide JB. 1981. Ecosystem theory and the unexpected: Implications for environmental toxicology. 43-73. In: BW Cornaby, ed, Management of Toxic Substances in Our Ecosystems. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Page AL, Miller RH, & Keeney DR, ed. 1982. Methods of Soil Analysis: Part 2: Chemical and Microbiological Properties. Second edition. American Society of Agronomy, Inc. and Soil Science Society of America, Inc., Madison, Wisconsin.

Parkinson D, Gray TR, & Williams ST. 1971. Methods for studying the ecology of soil micro-organisms. International Biological Program Handbook 19. Blackwell Scientific, Oxford, England. 115 p.

Phillipson, J. 1971. Methods of study in quantitative soil ecology, population, production and energy flow. International Biological Program Handbook 18. Blackwell Scientific, Oxford, England. 115 p.

Singh JS, & Gupta SR. 1977. Plant decomposition and soil respiration in terrestrial ecosystem. Botan. Rev. 43: 449-528.

Stotzky G. 1965. Microbial respiration, pp. 1550-1572. In: CA Black, ed. Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Madison, Wisconsin.

Tolle DA, Arthur MF, Van Voris P, Feder PI, Matthews MC, Chesson J, Larson ME, & Zwick TC. 1982. Evaluation of terrestrial microcosms for environmental studies of utility wastes. Third Ann. Prog. Rep. on RP 1224-5 to Electric Power Research Institute, Palo Alto, CA.

Van Voris P, O'Neill RV, Emanuel WR, & Shugart HH Jr,. 1980. Functional complexity and ecosystem stability. Ecology 61(6):1352-1360.

Van Voris P, Arthur MF, & Tolle DA. 1982. Evaluation of terrestrial microcosms for assessing ecological effects of utility wastes. EPRI Publication No. EA-2364. Electric Power Research Institute, Palo Alto, CA. 178 pp.

Van Voris P, Tolle DA, Arthur MF, Chesson J & Zwick TC. 1984. Development and Validation of Terrestrial Microcosm Test System for Assessing Ecological Effects of Utility Wastes. Final Project Report - RP1224-5. EPRI Publication No. EA-3672. Electric Power Research Institute, Palo Alto, CA. 192 pp.

Van Voris P, Tolle DA, Arthur MF, & Chesson J. 1985. Terrestrial Microcosms: validation, applications, and cost-benefit analysis. pp 117-142. In: J Cairns, Jr. ed. Multispecies Toxicity Testing. Pergamon Press, New York.

Zwick TC, Arthur MF, Tolle DA, & Van Voris P. 1984. A unique laboratory method for evaluating agro-ecosystem effects of an industrial waste product. Plant and Soil. 77: 395-399.